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Control of Glucose Concentration in a Fed-Batch Cultivation of *Scutellaria baicalensis G.* Plant Cells Using a Self-Organizing Fuzzy Logic Controller

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Abstract A self-organizing fuzzy logic controller using a genetic algorithm is described, which controlled the glucose concentration for the enhancement of flavonoid production in a fed-batch cultivation of *Scutellaria baicalensis G.* plant cells. The substrate feeding strategy in a fed-batch culture was to increase the flavonoid production by using the proposed kinetic model. For the two-stage culture, the substrate feeding strategy consisted of a first period with 28 g/l of glucose to promote cell growth, followed by a second period with 5 g/l of glucose to promote flavonoid production. A simple fuzzy logic controller and the self-organizing fuzzy logic controller using a genetic algorithm was constructed to control the glucose concentration in a fed-batch culture. The designed fuzzy logic controllers were applied to maintain the glucose concentration at given set-points of the two-stage culture in fed-batch cultivation. The experimental results showed that the self-organizing fuzzy logic controller improved the controller's performance, compared with that of the simple fuzzy logic controller. The specific production yield and productivity of flavonoids in the two-stage culture were higher than those in the batch culture.

Key words: *Scutellaria baicalensis G.*, flavonoids, fed-batch culture, self-organizing fuzzy control, genetic algorithm

The plant cell and tissue culture technique has been developed as an attractive alternative for the production of valuable compounds, in place of agricultural techniques [10]. The major valuable chemicals from plant cell culture are the secondary metabolites, which are biosynthetically derived from the primary metabolites [8]. *Scutellaria baicalensis G.* has been used as an active principle of herbal prescriptions for diseases such as inflammation and pyrexia, and its ether extract shows an augmentation of the cytotoxic effect of anticancer drugs against cancer cells [1]. *S. baicalensis G.* contains valuable flavonoid compounds such as baicalin and wogonin-7-0-glucuronic acid (GA). Cell culture of *S. baicalensis G.* has been economically successful to produce flavonoid compounds [22, 23].

To enhance production in a plant cell culture, a strategy for bioreactor operation and an optimized process should be developed via a bioprocess engineering [9]. A strategy is needed to optimize different stages related to cellular differentiation and secondary metabolite production by considering the characteristics of the plant cell culture. In this study, the two-stage culture as the strategy in a fed-batch operation was investigated to enhance the production of flavonoids in *S. baicalensis G.* plant cell culture. To perform the two-stage culture, the control of substrate concentration at two levels during the fed-batch culture should be done by an appropriate controller. The control of the plant cell culture process using classical controllers is very difficult, since it requires complicated, quantitative knowledge for the processes occurring under the influence of the control variables.

Since the relationship between state variables and control variables can not be determined by a simple function, and unpredictable probability factors exist in the cell culture process, a bioreactor operation usually depends on an expert's experience and imagination. Therefore, the cell culture process seems to be the most appropriate kind of process for applying a knowledge-based control instead of traditional control techniques [7, 19]. The fuzzy control system as knowledge-based control has been applied to control the substrate concentration in fermentations of microorganism [4, 28], yeast [2], and plant cell [5] culture.

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The simple fuzzy logic controller can be constructed, and its fuzzy rules are composed by the operator's experiences and knowledge of the cell culture process. However, the application of fuzzy control in a bioreactor operation has been limited. First, a reliable linguistic model of the operator's control strategy may not always be obtainable, and significant process changes may be beyond the operator's experience. Second, the design of a fuzzy control algorithm requires experience and information of the fuzzy control algorithm, which is generally unfamiliar to the biochemical engineer. In this study, a genetic algorithm [11] was applied to construct a self-organizing fuzzy logic controller for the control of substrate concentration in a fed-batch cultivation. In a self-organizing fuzzy logic controller, the genetic algorithm generates fuzzy rules along with the universe of discourse automatically. The designed fuzzy logic controller was applied to control the glucose concentration in a fed-batch culture of *S. baicalensis G.* plant cells. The performances of the simple and self-organizing fuzzy logic controllers were compared.

MATERIALS AND METHODS

Cell Line and Culture Method

Callus and suspension cultures of *S. baicalensis G.* cells were provided by Dr. W. T. Seo [22, 23]. Cell suspension cultures have been maintained on a modified Schenk and Hilldebrandt medium [20]. The pH of the medium was adjusted to 5.8 before autoclaving. After autoclaving, the pH of the medium was 5.6. Suspended cells were cultivated on a rotary shaker at 180 rpm and 25°C in the dark.

Analytical Methods

Using a sample port, a cell suspension of 25 ml was taken for analysis from a bioreactor. For the dry cell weight measurement, cell suspensions were filtered with dried and preweighted Whatman No. 1 filter paper with a slight vacuum. The fresh cell weight was measured using a balance as quickly as possible on a preweighted aluminum weighing tray. Filtered cells were washed with distilled water and dried in an oven to constant weight at 60°C for the dry weight measurement.

The quantitative concentration of flavonoids (baicalin and wogonin 7-O-GA) was determined by HPLC (ISCO 2350 HPLC pump with an V4 UV detector at 270 nm, Lincoln, Nebraska, U.S.A.) equipped with a stainless steel column (ISCO C18, 3.9×300 mm). One g of cells by fresh weight in 10 ml of methanol was sonicated at 125 W for 30 min. A filtered extract sample (10 µl) was injected into the HPLC system. The mobile phase consisted of 145 ml of tetrahydrofuran, 125 ml of 1,4-dioxane, 50 ml of methanol, 20 ml of acetic acid, 2 ml of 5% phosphoric acid, and 658 ml of deionized water. The flow rate of the mobile phase was 0.8 ml/min. Using a filtered sample of the medium, glucose concentrations were determined by the DNS (dinitrosalicylic acid) method [17] and with a glucose sensor (Boehringer Mannheim, Germany) simultaneously during the control experiments.

Bioreactor and Fuzzy Control System

For the bioreactor culture, cells in the late exponential growth phase, which were usually 5–6 days old, were used. About 40 g of cells by fresh weight was inoculated into a 2.5-l bioreactor (BIOFLO III, New Brunswick Sci., NJ, U.S.A.) containing 2 l of the medium. To culture *S. baicalensis G.* plant cells, the impeller and aeration systems in a bioreactor were modified. The impeller was designed as a hollow paddle type to minimize the shear rate [15, 26], since plant cells can not tolerate high-shear conditions and the mixing condition can cause a serious problem in a plant cell culture. Air supply into the culture was achieved via a silicon tube membrane (ID; 3 mm, OD; 4.5 mm) installed in the reactor wall [24].

Glucose feeding rate was controlled by the fuzzy logic controller. For control of the substrate feed rate in the fed-batch cultivation, the reactor was interfaced with a personal computer. The glucose concentration was detected at 6 h intervals and the data were transferred to the computer. The control output was calculated by the proposed control algorithm and the output signals were transferred to a peristaltic pump (Master flex, Model No. 752300, Cole-Parmer Inst. Co., U.S.A.) via a D/A converter, to manipulate the substrate feed rate.

Kinetic Model

To simulate the fuzzy rule generation, a simple kinetic model was developed as follows, based on experimental results of a batch cultivation of *S. baicalensis G.* As shown in Fig. 1, substrate utilization, cell growth, and flavonoid synthesis were monitored during 20 days of batch culture of *S. baicalensis G.*

It is assumed that cell growth is expressed by Monod kinetics and the rate of cell lysis is of the first order in a dead dry weight in which the cell death is considered. After the inoculation, lag phase is observed, which is required for cells to adapt to the change of its environment. In a cell culture of *S. baicalensis G.*, the lag time, t_L, is about one day. Mass balance of the dry cell weight can be expressed as

$$\frac{dX_d}{dt}=\left(1-\exp\left(-\frac{t}{t_L}\right)\right)\frac{\mu_{max}S}{K_D+S}X_d-k_DX_d \quad (1)$$

where X_d is dry cell weight (g/l), μ_{max} is maximum specific growth rate to the glucose source (g $g^{-1}d^{-1}$), S is glucose concentration (g/l), K_D is Monod constant (d^{-1}), and k_D is specific death rate (d^{-1}). The first term represents the cell

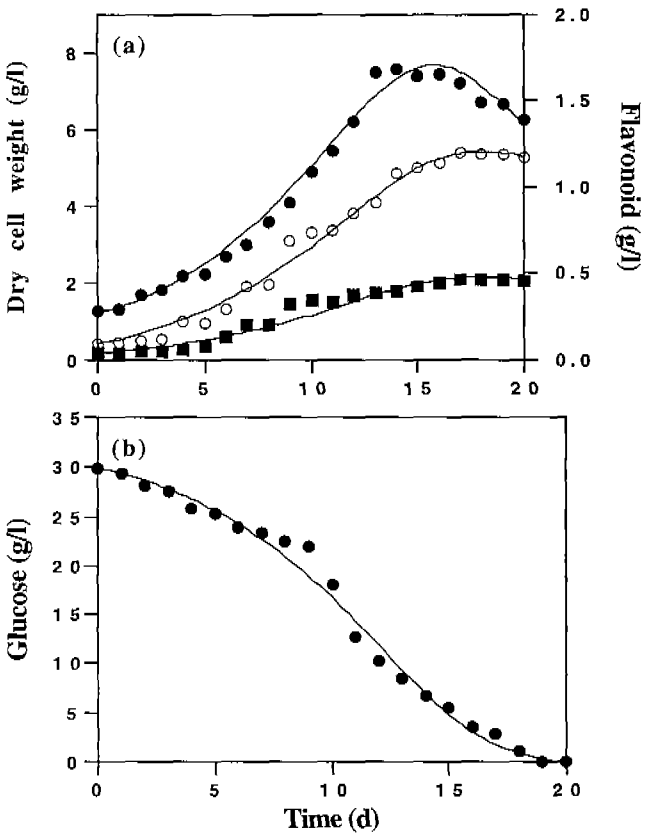


Fig. 1. Time course behavior of batch cultivation. (a) Dry cell weight and flavonoids. (b) Glucose.
Symbols: dry cell weight (●); baicalin (○); wogonin-7-0-GA (■); glucose (●); model prediction (solid line).

growth of glucose utilization and the second term represents the death of cell. The exponential term is used to express the lag time [18].

The specific production rate of flavonoids can be expressed as a combination of growth-associated term and nongrowth-associated term. It is assumed that the rate of product decomposition is of the first order in product concentration, when the product accumulation rate is influenced by the product decomposition [18]. The accumulation rate of flavonoids can be expressed as

$$\frac{dB}{dt}=\alpha_B\frac{dX_d}{dt}+\beta_B X_d - k_{PB}B \quad (2)$$

$$\frac{dW}{dt}=\alpha_W\frac{dX_d}{dt}+\beta_W X_d - k_{PW}W \quad (3)$$

where B is baicalin concentration (g/*l*), W is wogonin-7-0-GA concentration (g/*l*), α is a factor related to growth-associated production (dimensionless), β is a factor related to nongrowth associated production (d^{-1}), and k_p is rate constant of product degradation (d^{-1}). Subscripts B and W represent baicalin and wogonin-7-0-GA, respectively.

Table 1. List of the kinetic model parameters of the batch culture of *S. baicalensis G.*

Parameter	Dimension	Value
μ_{max}	$g\,g^{-1}d^{-1}$	0.327
K_G	g/l	13.522
k_{DL}	$g\,g^{-1}d^{-1}$	0.085
α_B	dimensionless	0.084
β_B	$g\,g^{-1}d^{-1}$	0.017
α_W	dimensionless	0.033
β_W	$g\,g^{-1}d^{-1}$	0.007
k_{PB}	$g\,g^{-1}d^{-1}$	0.072
k_{PW}	$g\,g^{-1}d^{-1}$	0.074

Glucose, as the carbon source, is utilized for cell growth and flavonoid (baicalin and wogonin-7-0-GA) formation. Therefore, the rate equation of glucose consumption can be expressed in terms of the consumption of cell growth and flavonoid production as

$$\frac{dS}{dt}=-\frac{1}{Y_{X/S}}\left(1-\exp\left(-\frac{t}{t_L}\right)\right)\frac{\mu_{max}S}{K_D+S}X_d - \frac{1}{Y_{B/S}}\left(\alpha_B\frac{dX_d}{dt}+\beta_B X_d\right)-\frac{1}{Y_{W/S}}\left(\alpha_W\frac{dX_d}{dt}+\beta_W X_d\right) \quad (4)$$

where $Y_{X/S}$, $Y_{B/S}$, and $Y_{W/S}$ are yield coefficients related to cell growth, baicalin production, and wogonin-7-0-GA production, respectively.

The parameters in the model equations are estimated by using a nonlinear parameter estimation technique [16, 21]. When the parameter estimation is completed, the model equations are solved simultaneously with numerical integration of differential equations by using a standard Runge-Kutta method. The experimental data were compared with the model predictions by choosing parameters which could provide a best fit of the model for the data. The estimated values of parameters are shown in Table 1. As shown in Fig. 1, the model could predict the experimental data with good agreement.

Substrate Feeding Strategy

Typical characteristics of plant cells in suspension culture are the slow cell growth rate and substrate uptake rate compared to those of microbes. In a batch suspension culture, *S. baicalensis G.* cells showed both a slow cell growth rate and substrate uptake rate, as shown in Fig. 1. Continuous feeding of the substrate in a continuous operation is not appropriate due to slow uptake rate of substrate and growth rate, and formation of aggregates of plant cell, which results in even larger volumes and effluence of unconsumed substrate. An approach to overcome the difficulties associated with continuous culture is the semicontinuous (fed-batch) culture. Since most secondary metabolites synthesis in the plant cell culture are stimulated

in the transition state, a fed-batch culture could be an attractive method [27]. As the strategy for the fed-batch culture, a single-stage culture and a two-stage culture have been used to produce the secondary metabolites in a plant cell culture [3, 5]. The single stage culture is an effective method to promote the cell growth and the production simultaneously, when product formation is growth-associated. In a two-stage culture method, the first stage is directed towards the rapid cell growth and the second stage is geared to product synthesis, where both the cell growth and the cell division are very low. In order to construct the feeding strategy, the effect of initial glucose concentration on the cell growth rate and the accumulation rate of flavonoids were studied with glucose concentrations of 1 to 30 g/l at 20 days of culture (Fig. 2). The cell growth rate was increased as glucose concentration increased, but the rate of increment over 28 g/l of glucose concentration was almost the same [Fig. 2(a)]. As shown in Fig. 2(b), the accumulation rate of flavonoids was a maximum at around 5 g/l of glucose concentration.

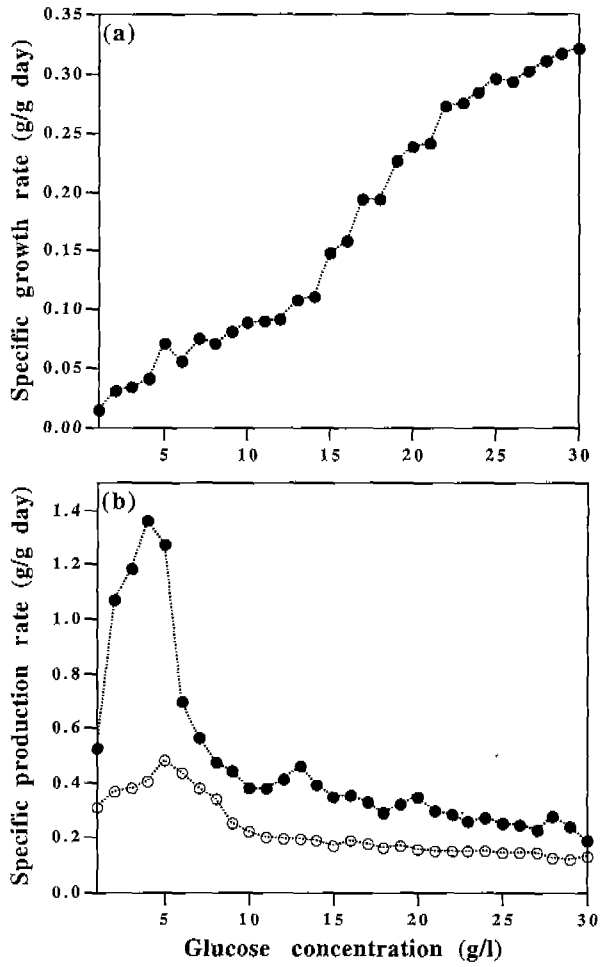


Fig. 2. Variation of specific cell growth rate and specific producion rate with respect to glucose concentration.
(a) Change of specific cell growth rate. (b) Change of specific production rate: baicalin (●); wogonin-7-0-GA (○).

Based on the result in Fig. 2, the substrate feeding strategy for the fed-batch culture as the two-stage culture was constructed as a single switch between two constant glucose concentrations. It should be noted that the two-stage fed-batch operation consists of the first period to promote cell growth, followed by the second period to promote the flavonoid production: In the first period, 28 g/l of glucose concentration was maintained to promote cell growth as well as flavonoid production. In the second period, 5 g/l of glucose concentration was maintained to promote flavonoid production, while the cell growth rate was kept in order to enhance flavonoid production and to prevent cell growth.

Self-Organizing Fuzzy Controller

Fuzzy controller. The fuzzy logic controller was designed to regulate the glucose concentration at a given set-point to increase the flavonoid synthesis in a *S. baicalensis G.* plant cell culture. The fuzzy control algorithm [25, 29] proceeded as follows; (1) fuzzification of fuzzy variables, error of substrate concentration, and change of error of substrate concentration using the membership function, (2) inference using fuzzified variables and the control rules by the min-max composition to obtain a fuzzy output, glucose feeding rate, and (3) defuzzification of glucose feeding rate by using the center of gravity method to obtain a numerical value.

As shown in Fig. 3, the self-organizing fuzzy controller using genetic algorithm was applied for the control of glucose concentration to increase the flavonoid production in a fed-batch culture of *S. baicalensis G.* Genetic algorithm was applied to construct the fuzzy rules by the modification of membership function and the modification of universe of discourse.

Fuzzy rules. While it was intended to apply the fuzzy control to a bioprocess, one of the key problems to be solved was to find fuzzy rules. For the fed-batch operation of *S. baicalensis G.*, the fuzzy controller consisted of two inputs and a single output. The input variables were identified as a set-point error of glucose concentration (E), and change in error of glucose concentration (CE). The control action (DU) was the change in glucose flow rate, to be applied to the process as an output variable. To improve the controller's performance, two more input variables (total cell dry weight and change in the total cell dry weight) should be added [5] but it was difficult to consider those variables due to the difficulty of on-line sensing in such practical applications. Although it was possible to do on-line sensing of the cell mass in a plant cell culture [6, 14], the *in situ* measurement of cell mass was not only very difficult but it had problem of measurement error. Consequently, by expressing such

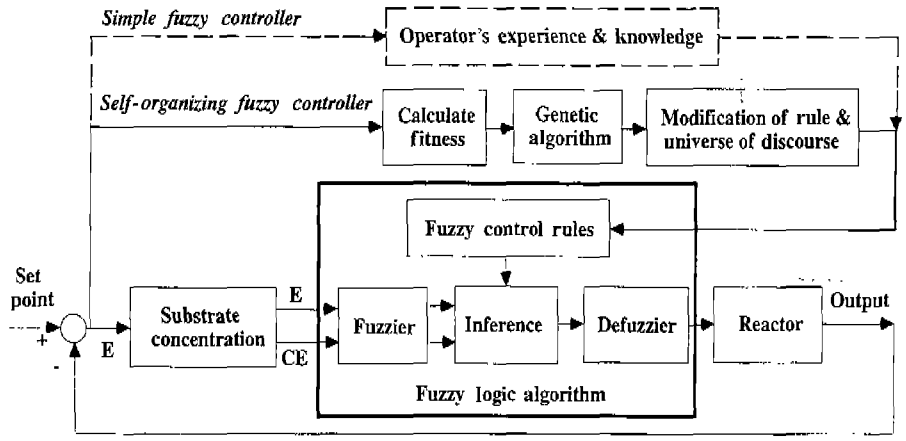


Fig. 3. Schematic diagram of the fuzzy logic controller (E: error; CE: change of error).

subjective evaluations represented by natural language as membership functions, control knowledge could be described as production rules having the following three fuzzy variables;

$$\text{Rule A if E is } E_i \text{ and CE is } CE_i \text{ then DU is } DU_i \quad (A=1\ldots N) \tag{5}$$

where, E_i, CE_i, and DU_i represent the linguistic labels of fuzzy variables E, CE, and DU, respectively. The labels consisted of the following: positive big, positive medium, positive small, zero, negative small, negative medium, negative big, abbreviated as PB, PM, PS, ZO, NS, NM and NB, respectively.

The composition of fuzzy rules for the simple fuzzy logic controller, consisting of N rules to be suitable for the control system, was carried out based on qualitative information, which was obtained from the batch culture experiment, as shown in Fig. 1. The adjustment of fuzzy rules for the self-organizing fuzzy controller, consisting of N rules, was completed by the modification of the membership function of antecedent and consequent in fuzzy conditional statements, and the universe of discourse. The membership function of consequent in fuzzy rules and the universe of discourse were modified for the optimization of fuzzy rule, which were automatically generated by a genetic algorithm, based on the simulation of a fed-batch culture using the proposed kinetic model. Figure 4 shows the scaling factor of universe of discourse expressed by a binary string, and the example of string composed of N membership functions. The optimization process was performed, based on genetic algorithm in cooperation with the represented string.

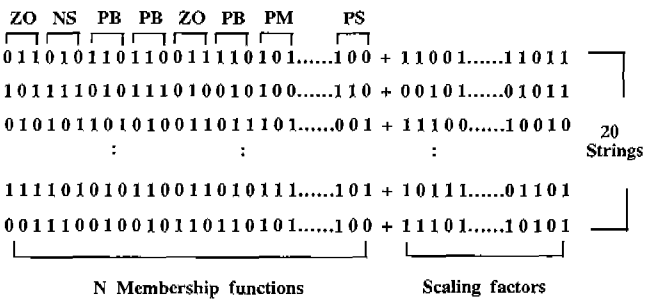


Fig. 4. String for the scaling factor and N membership functions.

Genetic algorithm: A genetic algorithm is recognized as a stochastic search method that is inspired by Darwinism evolution theory; natural evolution of selection by fitness [11-13]. It represents such variables in search of a binary coded string, which is referred to as a chromosome. In this study, fuzzy rules were converted to the binary coded string to be used as a chromosome. A population of chromosomes was prepared and their performances were evaluated by actually applying these binary encoded parameters to the objective system. The performance measure was a real number and this was referred to as a fitness value. The fitness function is shown in Eq. (6).

$$\text{Fitness}=\frac{K}{\sum_{i=1}^{n}|y_{set}-y_i|+\sum_{i=1}^{n}(y_{set}-y_i)^2} \tag{6}$$

where K represents the fitness constant, y_{set} is a set-point of substrate concentration, y_i is a substrate concentration, and n stands for number of generation. After the evaluation of each fitness value had been completed, all the chromosomes were combined with each other by genetic operators to reproduce a new set of chromosomes to be evaluated. Genetic operators were prepared with the following three variations: reproduction, crossover, and mutation. The overall process of the genetic algorithm for fuzzy rule generation and modification of universe of discourse is shown in Fig. 5.

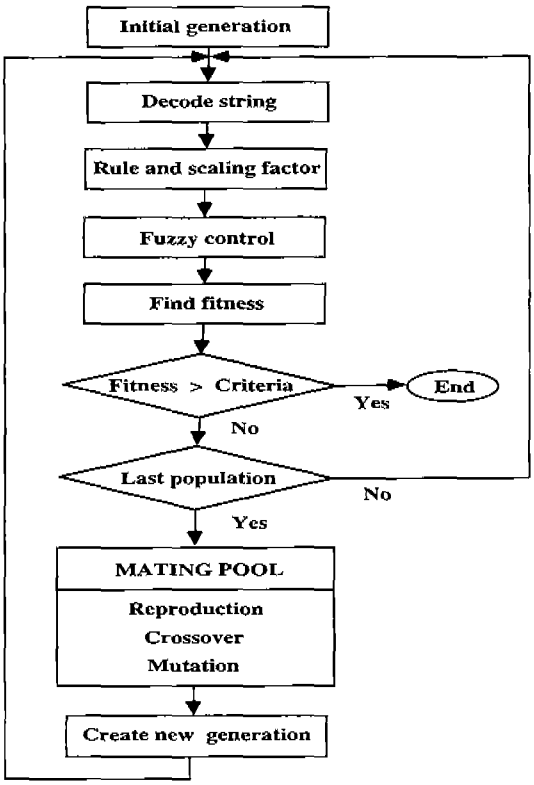


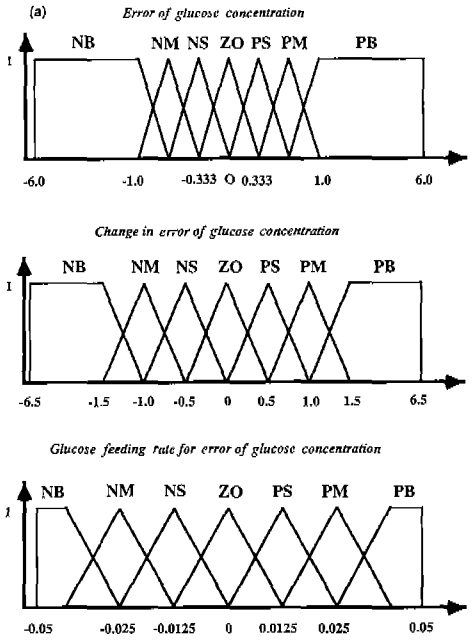
Fig. 5. Flow chart of the genetic algorithm.

RESULTS AND DISCUSSION

Two-Stage Fed-Batch Culture with Simple Fuzzy Logic Control

Fuzzy rules, which are based on the operator's inference, consist of 49 relations among the error of glucose concentration, the change in error of glucose concentration, and glucose feeding rate. Control output (glucose feeding rate) was determined in proportion to two input variables, the error of glucose concentration, and the change in error of glucose concentration. Membership functions and the fuzzy rule lookup table of glucose concentration are shown Figs. 6(a) and 6(b), respectively.

The experimental results in Fig. 7(a) represent the time course profile of the dry cell weight, the fresh cell weight, and glucose and flavonoid concentrations. The maximum dry cell weight was about 7.394 g/l at the 17th day, and the maximum flavonoid production of baicalin and wogonin-7-0-GA were 1.372 g/l and 0.504 g/l, respectively. Flavonoid production in the two-stage culture with simple fuzzy logic control was higher than those of the batch culture. Also, the specific production yield and the productivity of flavonoid production were increased. The control performance by a simple fuzzy logic controller is shown in Fig. 7(b). Although a simple fuzzy logic controller showed a fairly good control performance to keep the trajectory of glucose



(b)

CE / E	NB	NM	NS	ZO	PS	PM	PB
NB	NB	NB	NM	NM	NS	ZO	ZO
NM	NB	NM	NM	NS	ZO	ZO	ZO
NS	NM	NM	NS	ZO	ZO	PS	PS
ZO	NM	NS	ZO	ZO	ZO	PS	PM
PS	NS	ZO	ZO	ZO	PS	PM	PM
PM	ZO	ZO	PS	PS	PM	PM	PB
PB	ZO	ZO	PS	PM	PM	PB	PB

Fig. 6. (a) Membership functions and (b) fuzzy rule lookup table of a simple fuzzy logic controller.

concentration, little deviation from the set-point was observed. In the simulation of fed-batch culture using the proposed kinetic model, similar results were observed. The experimental and simulation results are summarized in Table 2.

Two-Stage Fed-Batch Culture with Self-Organizing Fuzzy Logic Control

Fuzzy rules and its parameters could be optimized by the genetic algorithm. The initial parameters for the genetic algorithm were as follows; population size, 20; length of individual string, 162; crossover rate, 1.0; and mutation rate, 0.03. Figure 8 shows the fitness value changes as generation increases. Its vertical axis is the inverse of the sum of error, and deviation from glucose set-point. The maximum, average, and minimum fitness were almost monotonically increasing in accordance with the generations. Maximum fitness was

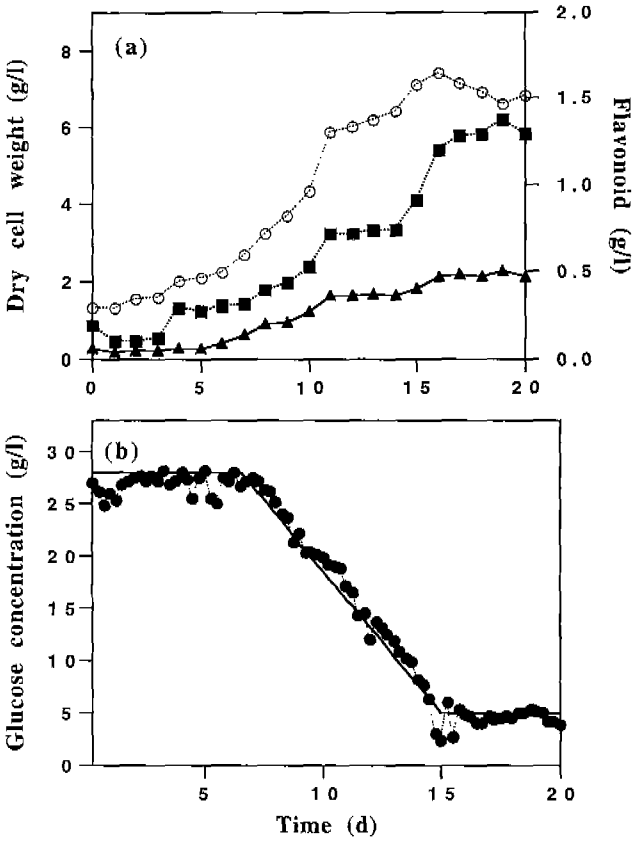


Fig. 7. Experimental results of fed-batch culture using a simple fuzzy logic controller. (a) Dry cell weight and flavonoid production; (b) glucose.
Symbols: dry cell weight (○); baicalin (■); wogonin-7-0-GA (▲); glucose (●); set-point (solid line).

achieved after the 40th generation, although little differences among the parameters for the maximum, average, and minimum fitness existed, and the population became saturated in their maximum values. So the differences should be considered as the drift caused by characteristics of the

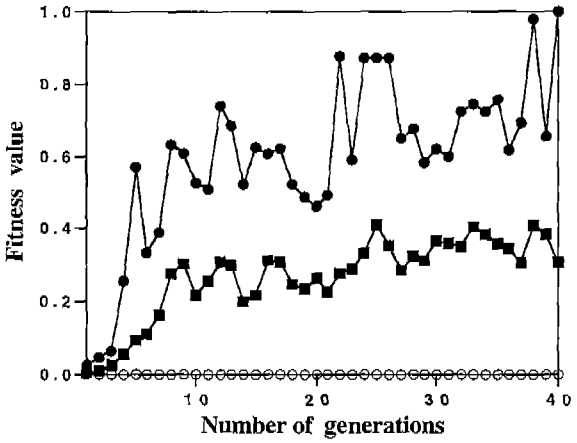


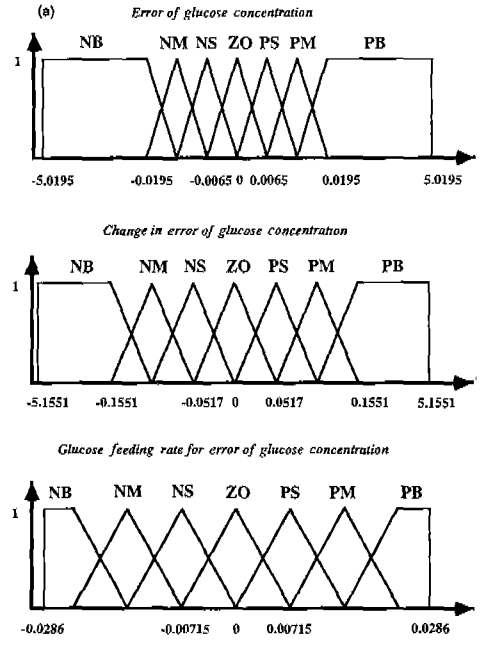
Fig. 8. Fitness value of individual generations.
Symbols: maximum fitness value (●); average fitness value (■); minimum fitness value (○).

proposed genetic algorithm. It could be concluded that the genetic algorithm could optimize the fuzzy rule base and its parameters in a short period of trials. The membership function and fuzzy control rules generated in the 40th generation were used to control the glucose concentration in the two-stage fed-batch culture. Fuzzy rules generated by the genetic algorithm consisted of 44 relations among the error of glucose concentration, the change in error of glucose concentration, and glucose feeding rate. Membership functions and the fuzzy rule lookup table of the glucose concentration are shown in Figs. 9(a) and 9(b), respectively.

The experimental results in Fig. 10 represent the time course profile of the dry cell weight, the fresh cell weight, and glucose and flavonoid production. The specific production yield and productivity were calculated. The maximum dry cell weight was about 8.723 g/l in the 13th day. The maximum production of baicalin and wogonin-7-0-GA in the 18th day were 1.639 g/l and 0.824 g/l, respectively. Flavonoid production in a two-stage culture

Table 2. Simulation and experimental results of batch and fed-batch culture of *S. baicalensis G.*

	Batch	Fed-Batch			
		Simple fuzzy control		Self-organizing fuzzy control	
	Experiment	Simulation	Experiment	Simulation	Experiment
Total Volume (l)	1.0000	1.1740	1.2140	1.1620	1.2250
Dry cell weight (g/l)	7.6100	9.2430	7.3940	9.2370	8.7230
Products (g/l)					
Baicalin	1.2000	1.5620	1.3720	1.5680	1.6390
Wogonin-7-0-GA	0.4670	0.5970	0.5040	0.5980	0.8240
Specific production yield (g/g DCW)					
Baicalin	0.1577	0.1690	0.1856	0.1698	0.1879
Wogonin-7-0-GA	0.0614	0.0646	0.0682	0.0647	0.0922
Productivity ($g\ l^{-1}d^{-1}$)					
Baicalin	0.0600	0.0781	0.0686	0.0784	0.0820
Wogonin-7-0-GA	0.0233	0.0298	0.0252	0.0299	0.0412



(b)

E \ CE	NB	NM	NS	ZO	PS	PM	PB
NB	NM	NM	NM	NS	PM	NM	PM
NM	ZO	PM	NS	PB	NS	NM	PM
NS	NM	NM	PS	PS	NS	ZO	NS
ZO	PB	PB	PM	PM	NM	ZO	NS
PS	NM	ZO	PS	NM	NB	NB	PS
PM	PS	ZO	NS	NS	ZO	PB	ZO
PB	NM	ZO	NS	NB	NM	NS	PM

Fig. 9. (a) Membership functions and (b) the fuzzy rule lookup table of self-organizing fuzzy logic controller.

with the self-organizing fuzzy logic controller was slightly higher than that in the two-stage culture with a simple fuzzy logic controller. Also, the specific production yield and the productivity of flavone glycosides were nearly the same. The control experimental result by the self-organizing fuzzy logic controller is shown in Fig. 10(b). Thus, the control performance of the self-organizing fuzzy logic controller improved over that of the simple fuzzy logic controller, which was based on the fitness value. As shown in Fig. 7(b) and Fig. 10(b), the fitness values for the simple fuzzy controller and self-organizing fuzzy logic controller, based on the deviation from a set-point, were 0.0049 and 0.0088, respectively. It could be concluded that the self-organizing fuzzy logic controller with improved control performance could be constructed using the genetic algorithm. The experimental and simulation results are summarized in Table 2.

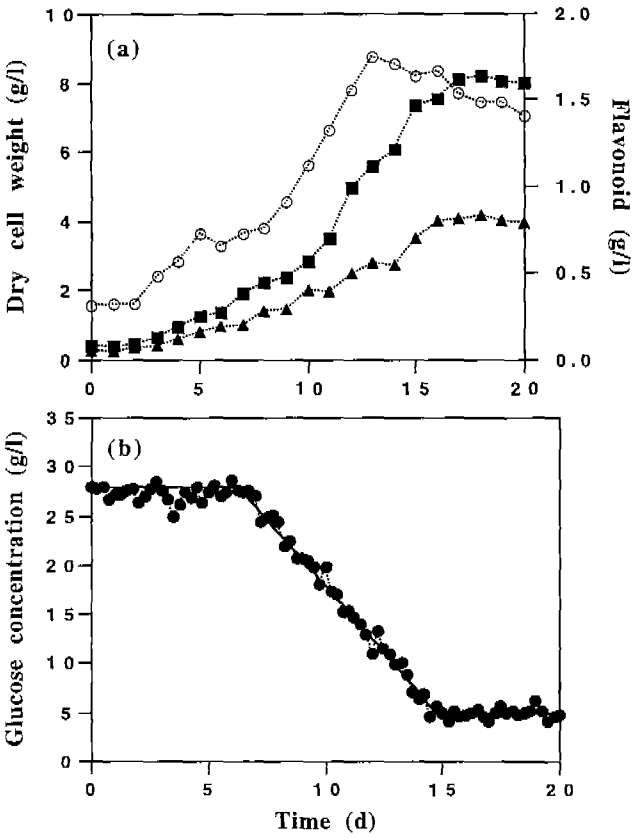


Fig. 10. Experimental results of fed-batch culture using the self-organizing fuzzy logic controller. (a) Dry cell weight and flavonoid production; (b) glucose.
Symbols: dry cell weight (○); baicalin (■); wogonin-7-0-GA (▲); glucose (●); set-point (solid line).

CONCLUSION

The proposed substrate feeding strategy in a fed-batch culture, the two-stage culture with the switching set-point of glucose concentration, enhanced the flavonoid production in *Scutellaria baicalensis G.* Fuzzy logic controllers were constructed to control the glucose concentration at a given set-point to increase the flavonoid production in a two-stage culture. A simple fuzzy logic controller based on the operator's inference and a self-organizing fuzzy logic controller using the genetic algorithm were constructed. According to the experimental results, a substrate concentration trajectory to increase flavone glycosides production in a two-stage culture could be successfully maintained by the fuzzy logic controller. In fact, the self-organizing fuzzy logic controller using the genetic algorithm actually improved the controller's performance due to the decrease of deviations from the set value. Also, the specific production yield and productivity of flavonoids in a two-stage culture were increased compared with those of the batch culture. Thus, the genetic algorithm could be applied to generate the fuzzy control

rules to control substrate concentration in a plant cell culture process. It could be concluded that the designed self-organizing fuzzy controller could generate fuzzy rules and automatically modify the universe of discourse in a fed-batch mode of bioprocess.

NOMENCLATURES

B : baicalin concentration (g/l)
K : fitness constant
K_D : Monod constant (g/l)
k_d : death rate constant (d^{-1})
k_p : product decomposition rate constant (d^{-1})
n : number of generation
S : glucose concentration (g/l)
t : time (d)
W : woginin-7-0-GA concentration (g/l)
X_d : dry cell weight (g/l)
$Y_{X/S}$: yield coefficient of glucose to ethanol (g/g)
$Y_{B/S}$: yield coefficient of glucose to baicalin (g/g)
$Y_{W/S}$: yield coefficient of glucose to wogonin-7-0-GA (g/g)
y_{set} : set-point of substrate concentration
y_i : substrate concentration

Greek Symbols

α : growth-associated production constant ($gg^{-1}d^{-1}$)
β : nongrowth-associated production constant ($gg^{-1}d^{-1}$)
μ : specific growth rate (d^{-1})

Subscripts

B : baicalin
L : lag phase
max : maximum
W : wogonin-7-0-GA

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